Expression analyses and characterization of isoprene synthase from *Populus alba*

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INTRODUCTION

Isoprene is a volatile C5 terpenoid that is released mainly from the leaves of many deciduous broad-leaved trees, such as *Salix*, *Quercus* and *Populus* species. The annual global emission of isoprene from these trees is estimated to be as high as 5 x 10^{14} g of carbon, which is similar to the level of methane, the most abundant naturally emitted hydrocarbon. Isoprene has been suggested to potentially provide general protection against environmental stress, such as heat and water as well as to protect against singlet oxygen.

To obtain biochemical and molecular biological insights into isoprene synthase, we cloned isoprene synthase cDNA from *P. alba* (PaIspS) and studied gene expression in response to environmental stress. Moreover, we examined the subcellular localization of PaIspS and also characterized its enzymatic function with a recombinant protein.

MATERIALS and METHODS

For the expression analyses of *PaIspS*, total RNA prepared from leaves, stems and roots were subjected to semi-quantitative RT-PCR. Expression level of the *PaIspS* was normalized by the *actin* values.

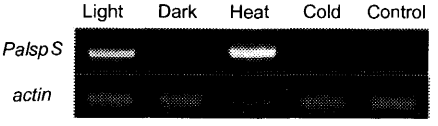
To analyze the subcellular localization of PaIspS, the nucleotide sequence for the putative transit peptide of PaIspS was subcloned into psmRS-GFP (accession number U70496) with a CaMV35S promoter. Onion peels and 1-month-old tobacco leaves were bombarded using a particle gun PDS-1000 according to the manufacturer's instructions. After 24 hr, GFP fluorescence of onion and tobacco cells was observed under a laser scanning confocal microscope.

To quantify the enzymatic activity of PaIspS, the sequence for the putative mature polypeptide of PaIspS was subcloned into pET22b and expressed in *Escherichia coli* origami B (DE3) with isopropyl β-D-thiogalactoside. Crude enzyme, which was prepared by sonication, was used for enzyme assay.

RESULTS and DISCUSSION

Isoprene synthase cDNA from *Populus alba* (PaIspS) was isolated by RT-PCR. This *PaIspS* mRNA, which was predominantly observed in the leaves, was strongly induced by heat stress and continuous light irradiation, and was substantially decreased in the dark, suggesting that isoprene emission was regulated at the transcriptional level (Fig.1). The subcellular localization of PaIspS protein with green fluorescent protein fusion was shown to be in plastids. PaIspS expressed in *E. coli* was characterized enzymatically: optimum pH of approximately 8.0, optimum temperature 40°C. Its preference for divalent cations for its activity was also studied. This optimum temperature is consistent with that the highest isoprene emission occurred between 30°C and 40°C.

Fig. 1 Expression analyses of *PaIspS* mRNA



Light, continuous light (170 μmol m^{-2} s^{-1}) at 25℃ for 24 hr; Dark, continuous dark at 25℃ for 24 hr; Heat, continuous light (120 μmol m^{-2} s^{-1}) at 40℃ for 6 hr; Cold, continuous light (120 μmol m^{-2} s^{-1}) at 15℃ for 6 hr; Control, 16 hr-light (120 μmol m^{-2} s^{-1}) / 8 hr-dark at 25℃ for 24 hr.

It has reported that temperature affects isoprene emission *in vivo*. The highest isoprene emission occurred between 30°C and 40°C. This observation is consistent with the transcriptional induction by heat stress and the optimum temperature of PaIspS determined in this study.

These results also suggest that its enzyme activity may be positively regulated under illumination in plastids because photosynthetic electron transport results in the accumulation of Mg^{2+} in the stroma, along with an increase in stromal pH. In addition to the transcriptional activation of *PaIspS* by light, this is advantageous for the production of isoprene under strong light conditions. In this study, it has indicated that the physiological function of emission of isoprene from plants is defense response against the heat and strong light stress.

REFERENCES

Sasaki, K., Ohara, K. and Yazaki, K. (2005) FEBS Lett. 579: 2514-2518